ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2006, p. 1347–1351 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1347–1351.2006

Involvement of the MexXY-OprM Efflux System in Emergence of Cefepime Resistance in Clinical Strains of *Pseudomonas aeruginosa*

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Received 12 August 2005/Returned for modification 2 October 2005/Accepted 29 January 2006

Cefepime (FEP) and ceftazidime (CAZ) are potent β-lactam antibiotics with similar MICs (1 to 2 μg/ml) for wild-type strains of *Pseudomonas aeruginosa*. However, recent epidemiological studies have highlighted the occurrence of isolates more resistant to FEP than to CAZ (FEP^r/CAZ^s profile). We thus investigated the mechanisms conferring such a phenotype in 38 clonally unrelated strains collected in two French teaching hospitals. Most of the bacteria (n = 32; 84%) appeared to stably overexpress the *mexY* gene, which codes for the RND transporter of the multidrug efflux system MexXY-OprM. MexXY up-regulation was the sole FEP resistance mechanism identified (n = 12) or was associated with increased levels of pump MexAB-OprM (n = 5) or MexJK (n = 2), synthesis of secondary β-lactamase PSE-1 (n = 10), derepression of cephalosporinase AmpC (n = 1), coexpression of both OXA-35 and MexJK (n = 1), or production of both PSE-1 and MexAB-OprM (n = 1). Down-regulation of the *mexXY* operon in seven selected strains by the plasmid-borne repressor gene *mexZ* decreased FEP resistance from two- to eightfold, thereby demonstrating the significant contribution of MexXY-OprM to the FEP^r/CAZ^s phenotype. The six isolates of this series that exhibited wild-type levels of the *mexY* gene were found to produce β-lactamase PSE-1 (n = 1), OXA-35 (n = 4), or both PSE-1 and OXA-35 (n = 1). Altogether, these data provide evidence that MexXY-OprM plays a major role in the development of FEP resistance among clinical strains of *P. aeruginosa*.

Cefepime (FEP) and ceftazidime (CAZ) are broad-spectrum cephalosporins that display similar MICs (1 to 2 μg/ml) for wild-type *Pseudomonas aeruginosa*. Both antibiotics have been approved for antipseudomonal chemotherapy (8) and are widely used to treat severely ill patients in hematology-oncology and intensive care units (13, 23, 38). Although MIC distribution patterns of both β-lactams (e.g., the MIC at which 50% of strains are inhibited [MIC_{50}]) appear to be identical for North American isolates of *P. aeruginosa* (14), several European studies have recently pointed out higher MIC_{50} values for cefepime (4 to 8 μg/ml) than for ceftazidime (2 to 4 μg/ml) (4–6, 39). These epidemiological data suggested the occurrence of isolates less susceptible to cefepime than to ceftazidime (FEP^r/CAZ^s phenotype).

Resistance of clinical strains of *P. aeruginosa* to antipseudomonal cephalosporins is mainly due to overexpression of the chromosomally encoded β-lactamase AmpC (with MICs of cefepime usually lower than those of ceftazidime) and occasionally to acquisition of extended-spectrum β-lactamases (41). However, while an increasing number of extended-spectrum β-lactamases have been characterized in ceftazidime-resistant isolates over the last decade (41), little information is available about β-lactamases that preferentially hydrolyze cefepime compared with ceftazidime. For instance, several FEP^r/CAZ^s strains were recently found to produce class D β-lactamases, such as OXA-1, OXA-10, OXA-31, and OXA-35 (3).

Cefepime may be a substrate for active efflux systems. In *P. aeruginosa*, several multidrug transporters, such as MexAB-OprM (34), MexCD-OprJ (33), and MexXY-OprM (29, 35), have been reported to accommodate zwitterionic β-lactams, such as cefepime and cefpirome (25). Whether these three pumps and some others described for *P. aeruginosa* (e.g., MexEF-OprN, MexGHI-OpmD, MexJK, and MexVW) may provide clinical strains with the FEP^r/CAZ^s phenotype has not been explored yet.

In the present study, we analyzed the mechanisms of a set of clonally unrelated FEP^r/CAZ^s isolates collected in two French teaching hospitals and showed that this phenotype is mostly due to stable overproduction of MexXY.

MATERIALS AND METHODS

Bacteria and drug susceptibility testing. Thirty-eight isolates of *P. aeruginosa* with higher resistance levels (MICs at least fourfold higher) to cefepime than to ceftazidime were isolated in 2003 in two French hospitals, namely, Bicêtre in Kremlin-Bicêtre (n = 16) and Jean Minjoz in Besançon (n = 22). These strains were genotyped by the random amplified polymorphic DNA technique (22) and found to be clonally unrelated (data not shown). The MICs of several antipseudomonal antibiotics were determined with the conventional dilution method in Mueller-Hinton agar medium (MHA; Bio-Rad, Ivry-sur-Seine, France) by using a Steers multiple inoculator and inocula of approximately 10^4 CFU per spot (32). The antimicrobial agents tested were kindly provided as titrated powders by GlaxoSmithKline (ceftazidime and ticarcillin; Marly-le-Roi, France), Bristol-Myers Squibb (aztreonam and cefepime; Paris, France), Wyeth-Lederle (piperacillin and tazobactam; Blois, France), Helm (apramycin; Hamburg, Germany), and Kyowa-Hakko-Kogyo (fortimicin; Tokyo, Japan).

Mutants that overproduce the efflux system MexXY were selected in vitro on MHA supplemented with cefepime (4 μg/ml) from two wild-type clinical strains of *P. aeruginosa* and from reference strain PAO1. Resistant clones developing after overnight incubation at 37°C were replicated on different MHA plates containing the MexXY substrates gentamicin (4 μg/ml) and ciprofloxacin (0.20 μg/ml), respectively. The MexXY gain-of-efflux mutants typically exhibited cross-resistance to aminoglycosides, ciprofloxacin, and cefepime. Mut-GR1, a PAO1 derivative with up-regulated pump MexXY, was used as a positive control for phenotypic comparisons (40).

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TABLE 1. Primers used for RT-PCR

Primer	5′→3′ nucleotide sequence	Reference
mexB1	ATC CGC CAG ACC ATC GCC A	This study
mexB2	CAT CAC CAG GAA CAC GAG GAG G	This study
mexC1	GTA CCG GCG TCA TGC AGG GTT	10
mexC2	TTA CTG TTG CGG CGC AGG TGA CT	10
mexE1	CCA GGA CCA GCA CGA ACT TCT TGC	10
mexE2	CGA CAA CGC CAA GGG CGA GTT CAC C	10
mexY1	TGG TCA ACG TCA GCG CCA GCT AT	10
mexY2	TCG ACG ATC TTC AGG CGG TTC TG	10
mexG1	GCA ACT GGC TCT GGC TGA CC	This study
mexG2	ACG GCG GTG GCG ATG TTG AA	This study
mexJ1	GCC CTG TCC CTG TTT TCC TCC C	This study
mexJ2	CCT TCT TTA CCC GCT CGC CG	This study
mexV1	CGT CAG CAG ATC GCC CTT TTC AGC	18
mexV2	CGC TTT TCG AGA TGG CCT TGC TGC	18

Prevalence of the FEP^r/CAZ^s resistance phenotype among clinical strains. The resistance patterns of 9,004 *P. aeruginosa* strains isolated in the hospital of Besançon between January 1999 and February 2005 were determined with the Kirby-Bauer disk method on Mueller-Hinton agar (32). A Sirscan automated image analyzer (I2A; Perols, France) was used to accurately measure the inhibition zones and to compute the resistance data (27). Bacterial susceptibility to cefepime and ceftazidime was determined by using disks loaded with 30 μg (Bio-Rad). Based on preliminary experiments with well-characterized strains (data not shown), isolates susceptible to ceftazidime (inhibition zone of ≥18 mm according to the NCCLS breakpoint of 8 μg/ml) (32) that showed inhibition zones to cefepime at least 4 mm smaller than to ceftazidime were recorded as FEP^r/CAZ^s positive.

Identification of β-lactamases. β-Lactamases of the isolates were released from culture extracts by ultrasonic treatment, and their pIs were determined by isoelectric focusing on a pH 3.5 to 10 ampholin polyacrylamide gel as described by Matthew et al. (26). Whole-cell DNA of *P. aeruginosa* was extracted as described previously (3). The DNA sequences of the PCR products were determined following cycle sequencing reactions (Big Dye terminator kit; Applied Biosystems, Foster City, CA) (9). Activities of chromosomally encoded cephalosporinase AmpC were determined spectrophotometrically in ultrasonic lysates by using nitrocefin as a chromogenic substrate (12).

Quantitative real-time PCR after RT-PCR. Expression of operons *mexAB-oprM*, *mexCD-oprJ*, *mexEF-oprN*, *mexXY*, *mexGHI-opmD*, *mexJK*, and *mexVW* was assessed by reverse transcription-PCR (RT-PCR) with specific primers (Table 1) by determining the transcript levels of the genes *mexB*, *mexC*, *mexE*, *mexY*, *mexG*, *mexJ*, and *mexV*, respectively, as described elsewhere (10, 12). PAO1 mutants overexpressing *mexB* (PT629 [10]), *mexC* (EryR [28]), *mexE* (PAO7H [15]), *mexY* (Mut-GR1 [40]), or *mexJ* (PAO318 [7]) served as controls and were analyzed in parallel with the FEP^r/CAZ^s clinical strains. Preliminary RT-PCR experiments on previously characterized clinical strains were performed to establish the cutoff values of expression for the identification of efflux overproducers in the FEP^r/CAZ^s collection. Six wild-type susceptible strains used as negative controls showed mRNA amounts of *mexB* (0.7 to 1.1 times) and *mexY* (1.2 to 2.6 times) very close to that of wild-type strain PAO1, respectively (Table 2). As expected, increased activities of *mexB* (2 to 12.4 times) and *mexY* (4 to 27.6 times) were detected in 12 other clinical strains known to overproduce both MexAB-OprM and MexXY (21) (Table 2). Based on these results, all the FEP^r/CAZ^s clinical strains with *mexB* expression at least two times, or *mexY* expression at least four times, higher than in PAO1 were considered MexAB-OprM and MexXY overproducers, respectively.

Nucleotide sequencing. The repressor gene *mexZ* and the *mexZ-mexX* intergenic region were amplified and sequenced as reported elsewhere (12).

Complementation with the *mexZ* gene. The *mexZ* gene of wild-type strain PAO1 has previously been cloned on broad-host-range vector pAK1900 (resistance marker to ampicillin and ticarcillin), yielding plasmid pAZ17 (40). Plasmids pAK1900 and pAZ17 were purified with the QIAGEN Midi kit and then transferred by electroporation (36) into seven FEP^r/CAZ^s clinical isolates. The resulting transformants were selected on MHA containing 250 or 500 μg/ml ticarcillin. Expression of *mexY* in the transformants (with pAZ17 or pAK1900) was assessed by RT-PCR.

TABLE 2. Expression of efflux genes in FEP^r/CAZ^s isolates of *P. aeruginosa*

	Specific mRNA level[a]		
	mexB	*mexY*	*mexJ*
Controls			
Wild-type efflux strain PAO1	1.0	1.0	1.0
Gain-of-efflux PAO1 mutants[b]	3.1	17.6	8.6
Wild-type efflux clinical strains (n = 6)	0.7–1.1	1.2–2.6	—[c]
Gain-of-efflux clinical strains (n = 12)	2–12.4	4–27.6	—
Selected FEP^r/CAZ^s strains (n = 38)			
Wild-type efflux isolates	0.6–1.5	1.2–2.8	0.5–3
Gain-of-efflux isolates[d]	2.1–3.6[e]	4–39[f]	5.1–5.5[g]

[a] Specific mRNAs were quantified by real-time PCR after retrotranscription. Values are means of two independent determinations and are expressed relative to PAO1, which was set at 1.
[b] Mutants PT629, MutGR1, and PAO318 were used as controls for MexAB-OprM, MexXY, and MexJK up-regulation, respectively.
[c] —, not determined.
[d] Defined as strains expressing *mexB* 2.0 times, *mexY* 4.0 times, or *mexJ* 5.0 times more than PAO1.
[e] n = 6.
[f] n = 32.
[g] n = 3.

RESULTS AND DISCUSSION

Involvement of the efflux system MexXY in the FEP^r/CAZ^s phenotype. Quantitative RT-PCR analysis of 38 genotypically distinct FEP^r/CAZ^s clinical isolates of *P. aeruginosa* revealed a strong proportion of strains (n = 32; 84%) overexpressing gene *mexY* (from 4- to 39-fold) that codes for the RND transporter MexY, compared with wild-type susceptible strain PAO1 (Table 2). In agreement with an increased drug efflux in these 32 isolates, all of them exhibited reduced susceptibility to various antibiotics known to be substrates of the MexXY-OprM pump (25, 35), including enzyme-recalcitrant aminoglycosides apramycin (MICs 2- to 8-fold higher than that for PAO1) and fortimicin (4- to 16-fold higher) (Table 3).

Contribution of efflux pumps to β-lactam resistance may be masked by synthesis of secondary β-lactamases or derepression of cephalosporinase AmpC (31). Interestingly, 12 of the 32 MexXY-overproducing isolates reported above turned out to contain only basal amounts of AmpC, like in PAO1 (data not shown). These 12 strains were four- to eightfold more resistant to cefepime (MICs of 8 to 16 μg/ml) than to ceftazidime (MICs of 1 to 4 μg/ml) (Table 3). Confirming a major role of MexXY in the FEP^r/CAZ^s phenotype, complete or partial down-regulation of operon *mexXY* in five selected isolates by plasmid-encoded repressor MexZ (construct pAZ17) reversed cefepime resistance to wild-type levels (Table 4). It should also be noted that, consistent with previous observations (21, 37), the resistance levels (MICs) to cefepime, apramycin, or fortimicin were not strictly proportional to that of the *mexY* transcripts in the FEP^r/CAZ^s strains. For instance, strains with different *mexY* activities (4- and 39-fold that of reference PAO1, for example) exhibited similar resistance to these antibiotics. Thus, while RT-PCR tends to be the method of choice to characterize gain-of-efflux mutants in *P. aeruginosa* (10, 21, 43), it cannot predict the degree of resistance conferred by pumps, likely because of interference of complex

TABLE 3. Antibiotic resistance of FEP^r/CAZ^s strains according to the resistance mechanisms involved

Strain	Resistance mechanism(s)[a]	MIC (μg/ml)[d]						
		FEP	CAZ	TIC	ATM	TZP	APR	FOR
Reference strains[b]								
PAO1	Wild type	2	2	16	4	4	4	16
Mut-GR1	XY	8	2	16	4	—[c]	16	64
PT629	ABM	4	4	64	16	—	4	16
ATM4	ABM, XY	16	4	64	16	—	—	—
Clinical strains (no. with mechanism[s])								
12	XY	8–16	1–4	—	—	—	8–16	64–256
5	XY, ABM	16–32	4	64–128	16–32	16	8–16	128
2	XY, JK	8	2	—	—	—	8–16	128
1	PSE-1	8	2	>256	8–32	64–128	—	—
6	XY, PSE-1	8–32	2–4	>256	8–16	8–128	8–32	64–256
4	OXA-35	16–32	1–4	>256	4–16	64–128	—	—
1	XY, JK, OXA-35	32	4	>256	16	128	8	64
4	XY, PSE-1 (OXA-9)	16–32	2–4	>256	8–16	32–128	8–32	64–128
1	PSE-1, OXA-35 (OXA-9)	32	8	>256	32	128	—	—
1	XY, AmpC	32	8	128	16	16	16	128
1	XY, ABM, PSE-1 (OXA-9)	64	4	>256	64	128	8	128

[a] Overproduction of pumps MexXY (XY), MexAB-OprM (ABM), and MexJK (JK); production of β-lactamases PSE-1, OXA-35, and OXA-9; or stable overexpression of cephalosporinase AmpC (AmpC).
[b] Mut-GR1 (40), PT629 (10) and ATM4 (21).
[c] —, not determined.
[d] Abbreviations: TIC, ticarcillin; ATM, aztreonam; TZP, piperacillin-tazobactam; APR, apramycin; FOR, fortimicin.

factors modulating the pump efficacy (e.g., membrane permeability to pump substrates, drug target alterations, and alterations in the tripartite system itself).

Resistance by efflux in clinical strains of *P. aeruginosa* has been associated with the occurrence of mutations inside or outside the regulatory gene *mexZ* (*agrZ* or *agrW* mutants, respectively) (21) that controls the expression of the operon *mexXY*. Contrasting with previous conclusions on non-cystic fibrosis isolates (37, 40), DNA sequencing experiments on seven MexXY overproducers of the FEP^r/CAZ^s collection identified most of them (five out of seven) as *agrZ* and not *agrW* mutants (Table 4). No mutation could be detected in the *mexZ-mexX* intergenic region of these bacteria.

Double efflux mutants. Five additional FEP^r/CAZ^s strains that showed no significant β-lactamase production were found to overexpress operons *mexAB-oprM* and *mexXY* concomitantly, with *mexB* levels 2.1- to 3.6-fold higher than those observed in PAO1 (Table 2). Their moderate resistance to ticarcillin (MIC, 64 to 128 μg/ml) and aztreonam (MIC, 16 to 32 μg/ml) is typical of that exhibited by MexAB-OprM-overproducing mutants (44) (Table 3). In agreement with recent data (21), simultaneous overexpression of MexXY and MexAB-

TABLE 4. Influence of plasmid-encoded MexZ (pAZ17) on resistance level to cefepime

Strain (plasmid)	Initial resistance phenotype[a] (*agr* genotype)	*mexZ*[b]	*mexY* expression	FEP MIC[d] (μg/ml)
PAO1	Wild type		1.0	2
PAO1 (pAK1900)			0.8	2
PAO1 (pAZ17)			0.1	2
46 (pAK1900)	XY (*agrZ*)	CTC→CGA (L_{138}R)	7.4	8
46 (pAZ17)			3.7	2
72 (pAK1900)	XY (*agrZ*)	6 bp inserted between nt 166 and 167	27.2	8
72 (pAZ17)			1.3	2
105 (pAK1900)	XY (*agrZ*)	1 bp inserted between nt 182 and 183	74.6	16
105 (pAZ17)			17.8	2
34 (pAK1900)	XY (*agrZ*)	TGG→TCG (W_{176}S)	17.9	8
34 (pAZ17)			0.1	2
100 (pAK1900)	XY (*agrW*)		19.3	8
100 (pAZ17)			0.9	2
2030 (pAK1900)	XY (*agrW*), AB		6.1	8
2030 (pAZ17)			0.1	4
60 (pAK1900)	XY (*argZ*), AmpC	GGC→GAA (G_{195}E)	54.6	32
60 (pAZ17)			13.5	16

[a] See footnotes for Table 3.
[b] Nucleotide (nt) differences with the PAO1 genome (www.pseudomonas.com).
[c] Relative to PAO1; mean values from two independent experiments.
[d] Determination of the cefepime MIC was done in the presence of 100 μg/ml of ticarcillin to maintain pAK1900 and pAZ17 plasmids.

OprM in these isolates resulted in a modest twofold increase in resistance to cefepime (MIC, up to 32 μg/ml), which is a substrate for both pumps (17, 25), compared with single MexXY deregulation (MICs, up to 16 μg/ml) (Table 3) (16). The additive effect of MexXY on cefepime resistance in these double efflux mutants was confirmed by turning down *mexXY* expression. Introduction of the plasmid-borne *mexZ* gene (plasmid pAZ17) in a selected isolate, named 2030, indeed reduced the MIC of cefepime (4 μg/ml) to that for the MexAB-OprM-overproducing control, PT629 (Table 4). As already noticed (21), the impact of the MexXY-OprM pump on the cefepime MIC was low (twofold increase) when coexpressed with MexAB-OprM.

Given that cefepime is a good substrate for the efflux system MexCD-OprJ (25), we determined the transcript levels of gene *mexC* by RT-PCR in the 38 FEP^r/CAZ^s isolates. Expression of *mexC* was far below (<10%) that of MexCD-OprJ gain-of-efflux mutant EryR (expressing *mexC* 240 times more than PAO1 [data not shown]), demonstrating the absence of MexCD-OprJ overproducers in the present collection. Interestingly, a systematic search for MexCD-OprJ up-regulated mutants among the strains of *P. aeruginosa* routinely isolated at the hospital of Besançon led us to the conclusion that these mutants are rather infrequent in the clinical setting, maybe because of their reduced virulence (19) (unpublished data).

Negative results for the FEP^r/CAZ^s isolates were obtained with the genes *mexE*, *mexG*, and *mexV* used as representatives of operons *mexEF-oprN* (15), *mexGHI-opmD* (1), and *mexVW* (18), respectively (data not shown). Of note, three MexXY overproducers were shown to simultaneously express *mexJ* at levels close to that of the MexJK gain-of-efflux mutant PAO318 (Table 2). With the exception of one strain producing the enzyme OXA-35 (see below), these double gain-of-efflux mutants were as susceptible to cefepime as single MexXY producers (Table 3), suggesting that this antibiotic is not a substrate for the MexJK-OprM/OpmH pump.

Single β-lactamase production. Of the six FEP^r/CAZ^s strains showing wild-type basal pump expression, one produced β-lactamase PSE-1, four produced OXA-35, an enzyme closely related to OXA-10 and OXA-13 (2), and the other one produced simultaneously the PSE-1, OXA-35, and OXA-9 enzymes (Table 3). PSE-1 and OXA-35 are known to confer high resistance to ticarcillin and piperacillin and to provide low-level resistance to aztreonam and cefepime (2, 20).

Combination of β-lactamase and efflux mechanisms. Production of the enzymes PSE-1 or OXA-35 in combination with MexXY up-regulation, as observed in six and one strains (the latter also overexpressing *mexJK*), respectively, had limited cumulative effects on the resistance levels to cefepime (MICs, 8 to 32 μg/ml) (Table 3). This result is reminiscent of the observation that increased efflux does not really impact the resistance levels to β-lactams conferred by β-lactamases in clinical (12) or laboratory (31) strains. Similarly, plasmid pAZ17-promoted repression of *mexXY* in a double AmpC/MexXY-overproducing strain (named 60) that resulted in only a twofold reduction in the cefepime MIC (Table 4). The reasons for this lack of cooperativity between the two mechanisms remain unclear. A plausible hypothesis would be that both the intact and cleaved β-lactam molecules compete for binding at the active sites at MexB or MexY, thus decreasing the transport efficacy of corresponding pumps.

The narrow-spectrum β-lactamase OXA-9 was found in six isolates, but its contribution to cefepime resistance is likely to be negligible, as deduced from the comparison of the MICs of cefepime for strains coexpressing PSE-1 and MexXY with or without OXA-9 (Table 3). Interestingly, the highest resistance level to cefepime (MIC, 64 μg/ml) was reached by an isolate combining three resistance mechanisms, namely, the increased expression of efflux systems MexXY and MexAB-OprM together with production of β-lactamase PSE-1.

Prevalence of the FEP^r/CAZ^s phenotype among clinical isolates. A retrospective study on 9,004 consecutive strains of *P. aeruginosa* isolated at the hospital of Besançon from 1999 to 2005 found a notable proportion (32.7%) of FEP^r/CAZ^s isolates, without any temporal increase over the studied period. Reinforcing the notion that most of these bacteria are gain-of-efflux mutants, we observed recently that 40 out of 105 (38.1%) bacteremic *P. aeruginosa* isolates collected in 1999 were MexXY overproducers (D. Hocquet, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2838, 2002).

To get some insight into the role of cefepime in the emergence of this type of efflux mutants, we carried out in vitro experiments with reference strain PAO1 and two wild-type clinical strains. Cefepime, at a concentration of 4 μg/ml, could select mutants with a typical MexXY resistance profile at rates ranging from 2.5×10^{-8} to 6×10^{-7} (data not shown).

Conclusion. This study strongly suggests that the FEP^r/CAZ^s resistance phenotype commonly observed among the French strains of *P. aeruginosa* is primarily due to stable overexpression of the efflux system MexXY. Whether therapeutic use of cefepime may have promoted such a resistance profile is still unclear. Indeed, while this antibiotic has been shown to readily select for MexXY-overproducing mutants in vitro, its low consumption at the hospital of Besançon (less than 1% of the whole defined daily doses of antibiotics prescribed) seems to preclude a major contribution to the high prevalence of the FEP^r/CAZ^s isolates recorded locally. Since aminoglycosides, alone or in combination with fluoroquinolones, may also select for MexXY gain-of-efflux mutants in vitro (24, 40, 42), we have recently initiated a time series analysis (30) to determine the potential relationship between antibiotics use and occurrence of FEP^r/CAZ^s strains.

Pharmocokinetic-pharmacodynamic studies have suggested that low resistance levels to cefepime and fluoroquinolones, as those exhibited by MexXY overproducers, might be therapeutically significant and associated with poor clinical outcome (P. G. Ambrose, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1020, 2002) (11). Accordingly, a prudent attitude for clinicians would be to avoid whenever possible the use of these antibiotics to treat infections caused by MexXY efflux mutants.

ACKNOWLEDGMENTS

F.E.G. was supported by a grant from the French association "Vaincre La Mucoviscidose." This work was also funded by a grant from the European Community (6th PCRD, LSHM-CT-2003-503335).

We thank Florence Giachetti for her excellent technical assistance.

REFERENCES

1. **Aendekerk, S., B. Ghysels, P. Cornelis, and C. Baysse.** 2002. Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. Microbiology **148:**2371–2381.
2. **Aubert, D., L. Poirel, A. B. Ali, F. W. Goldstein, and P. Nordmann.** 2001.

OXA-35 is an OXA-10-related β-lactamase from *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **48:**717–721.
3. **Aubert, D., L. Poirel, J. Chevalier, S. Leotard, J. M. Pages, and P. Nordmann.** 2001. Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **45:**1615–1620.
4. **Blandino, G., A. Marchese, F. Ardito, G. Fadda, R. Fontana, G. Lo Cascio, F. Marchetti, G. C. Schito, and G. Nicoletti.** 2004. Antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated in Italy from patients with hospital-acquired infections. Int. J. Antimicrob. Agents **24:**515–518.
5. **Bonfiglio, G., and F. Marchetti.** 2000. *In vitro* activity of ceftazidime, cefepime and imipenem on 1,005 *Pseudomonas aeruginosa* clinical isolates either susceptible or resistant to beta-lactams. Chemotherapy **46:**229–234.
6. **Cavallo, J. D., R. Fabre, F. Leblanc, M. H. Nicolas-Chanoine, and A. Thabaut.** 2000. Antibiotic susceptibility and mechanisms of β-lactam resistance in 1310 strains of *Pseudomonas aeruginosa:* a French multicentre study (1996). J. Antimicrob. Chemother. **46:**133–136.
7. **Chuanchuen, R., C. T. Narasaki, and H. P. Schweizer.** 2002. The MexJK efflux pump of *Pseudomonas aeruginosa* requires OprM for antibiotic efflux but not for efflux of triclosan. J. Bacteriol. **184:**5036–5044.
8. **Craig, W. A., and S. C. Ebert.** 1994. Antimicrobial therapy in *Pseudomonas aeruginosa* infections, p. 441–517. *In* A. L. Baltch and R. P. Smith (ed.), *Pseudomonas aeruginosa* infections and treatment. Marcel Bekker, Inc., New York, N.Y.
9. **De Champs, C., L. Poirel, R. Bonnet, D. Sirot, C. Chanal, J. Sirot, and P. Nordmann.** 2002. Prospective survey of β-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolated in a French hospital in 2000. Antimicrob. Agents Chemother. **46:**3031–3034.
10. **Dumas, J.-L., C. Delden, K. Perron, and T. Köhler.** 2006. Analysis of antibiotic resistance gene expression in *Pseudomonas aeruginosa* by quantitative real-time-PCR. FEMS Microbiol. Lett. **254:**217–225.
11. **Dupont, P., D. Hocquet, K. Jeannot, P. Chavanet, and P. Plésiat.** 2005. Bacteriostatic and bactericidal activities of eight fluoroquinolones against MexAB-OprM-overproducing clinical strains of *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **55:**518–522.
12. **Hocquet, D., X. Bertrand, T. Kohler, D. Talon, and P. Plésiat.** 2003. Genetic and phenotypic variations of a resistant *Pseudomonas aeruginosa* epidemic clone. Antimicrob. Agents Chemother. **47:**1887–1894.
13. **Hughes, W. T., D. Armstrong, G. P. Bodey, E. J. Bow, A. E. Brown, T. Calandra, R. Feld, P. A. Pizzo, K. V. Rolston, J. L. Shenep, and L. S. Young.** 2002. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin. Infect. Dis. **34:**730–751.
14. **Karlowsky, J. A., M. E. Jones, C. Thornsberry, A. T. Evangelista, Y. C. Yee, and D. F. Sahm.** 2005. Stable antimicrobial susceptibility rates for clinical isolates of *Pseudomonas aeruginosa* from the 2001–2003 tracking resistance in the United States today surveillance studies. Clin. Infect. Dis. **40**(Suppl. 2)**:**S89–S98.
15. **Köhler, T., M. Michéa Hamzehpour, U. Henze, N. Gotoh, L. Kocjancic Curty, and J.-C. Pechère.** 1997. Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. Mol. Microbiol. **23:**345–354.
16. **Lee, A., W. Mao, M. S. Warren, A. Mistry, K. Hoshino, R. Okumura, H. Ishida, and O. Lomovskaya.** 2000. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. J. Bacteriol. **182:**3142–3150.
17. **Li, X. Z., H. Nikaido, and K. Poole.** 1995. Role of MexA-MexB-OprM in antibiotic efflux in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **39:**1948–1953.
18. **Li, Y., T. Mima, Y. Komori, Y. Morita, T. Kuroda, T. Mizushima, and T. Tsuchiya.** 2003. A new member of the tripartite multidrug efflux pumps, MexVW-OprM, in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **52:**572–575.
19. **Linares, J. F., J. A. Lopez, E. Camafeita, J. P. Albar, F. Rojo, and J. L. Martinez.** 2005. Overexpression of the multidrug efflux pumps MexCD-OprJ and MexEF-OprN is associated with a reduction of type III secretion in *Pseudomonas aeruginosa*. J. Bacteriol. **187:**1384–1391.
20. **Livermore, D. M.** 1995. β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. **8:**557–584.
21. **Llanes, C., D. Hocquet, C. Vogne, D. Bénali-Baitich, C. Neuwirth, and P. Plésiat.** 2004. Clinical strains of *Pseudomonas aeruginosa* overproducing simultaneously MexAB-OprM and MexXY efflux pumps. Antimicrob. Agents Chemother. **48:**1797–1802.
22. **Mahenthiralingam, E., M. E. Campbell, J. Foster, J. S. Lam, and D. P. Speert.** 1996. Random amplified polymorphic DNA typing of *Pseudomonas aeruginosa* isolates recovered from patients with cystic fibrosis. J. Clin. Microbiol. **34:**1129–1135.
23. **Mandell, L. A., J. G. Bartlett, S. F. Dowell, T. M. File, Jr., D. M. Musher, and C. Whitney.** 2003. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin. Infect. Dis. **37:**1405–1433.
24. **Masuda, N., E. Sakagawa, S. Ohya, N. Gotoh, H. Tsujimoto, and T. Nishino.** 2000. Contribution of the MexX-MexY-OprM efflux system to intrinsic resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**2242–2246.
25. **Masuda, N., E. Sakagawa, S. Ohya, N. Gotoh, H. Tsujimoto, and T. Nishino.** 2000. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-OprM efflux pumps in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**3322–3327.
26. **Mathew, A., A. M. Harris, M. J. Marshall, and G. W. Ross.** 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. **88:**169–178.
27. **Medeiros, A. A., and J. Crellin.** 2000. Evaluation of the Sirscan automated zone reader in a clinical microbiology laboratory. J. Clin. Microbiol. **38:**1688–1693.
28. **Michéa Hamzehpour, M., J. C. Pechère, P. Plésiat, and T. Köhler.** 1995. OprK and OprM define two genetically distinct multidrug efflux systems in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **39:**2392–2396.
29. **Mine, T., Y. Morita, A. Kataoka, T. Mizushima, and T. Tsuchiya.** 1999. Expression in *Escherichia coli* of a new multidrug efflux pump MexXY, from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:**415–417.
30. **Muller, A., J. M. Lopez-Lozano, X. Bertrand, and D. Talon.** 2004. Relationship between ceftriaxone use and resistance to third-generation cephalosporins among clinical strains of *Enterobacter cloacae*. J. Antimicrob. Chemother. **54:**173–177.
31. **Nakae, T., A. Nakajima, T. Ono, K. Saito, and H. Yoneyama.** 1999. Resistance to β-lactam antibiotics in *Pseudomonas aeruginosa* due to interplay between the MexAB-OprM efflux pump and β-lactamase. Antimicrob. Agents Chemother. **43:**1301–1303.
32. **National Committee for Clinical Laboratory Standards.** 1997. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, vol. 17, no. 2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
33. **Poole, K., N. Gotoh, H. Tsujimoto, Q. Zhao, A. Wada, T. Yamasaki, S. Neshat, J. Yamagishi, X. Z. Li, and T. Nishino.** 1996. Overexpression of the *mexC-mexD-oprJ* efflux operon in *nfxB*-type multidrug-resistant strains of *Pseudomonas aeruginosa*. Mol. Microbiol. **21:**713–724.
34. **Poole, K., K. Krebes, C. McNally, and S. Neshat.** 1993. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J. Bacteriol. **175:**7363–7372.
35. **Ramos Aires, J., T. Köhler, H. Nikaido, and P. Plésiat.** 1999. Involvement of an efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob. Agents Chemother. **43:**2624–2628.
36. **Smith, A. W., and B. H. Iglewski.** 1989. Transformation of *Pseudomonas aeruginosa* by electroporation. Nucleic Acids Res. **17:**10509.
37. **Sobel, M. L., G. A. McKay, and K. Poole.** 2003. Contribution of the MexXY multidrug transporter to aminoglycoside resistance in *Pseudomonas aeruginosa* clinical isolates. Antimicrob. Agents Chemother. **47:**3202–3207.
38. **Solomkin, J. S., J. E. Mazuski, E. J. Baron, R. G. Sawyer, A. B. Nathens, J. T. DiPiro, T. Buchman, E. P. Dellinger, J. Jernigan, S. Gorbach, A. W. Chow, and J. Bartlett.** 2003. Guidelines for the selection of anti-infective agents for complicated intra-abdominal infections. Clin. Infect. Dis. **37:**997–1005.
39. **Van Eldere, J.** 2003. Multicentre surveillance of *Pseudomonas aeruginosa* susceptibility patterns in nosocomial infections. J. Antimicrob. Chemother. **51:**347–352.
40. **Vogne, C., J. R. Aires, C. Bailly, D. Hocquet, and P. Plésiat.** 2004. Role of the multidrug efflux system MexXY in the emergence of moderate resistance to aminoglycosides among *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother. **48:**1676–1680.
41. **Weldhagen, G. F., L. Poirel, and P. Nordmann.** 2003. Ambler class A extended-spectrum β-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. Antimicrob. Agents Chemother. **47:**2385–2392.
42. **Westbrock-Wadman, S., D. R. Sherman, M. J. Hickey, S. N. Coulter, Y. Q. Zhu, P. Warrener, L. Y. Nguyen, R. M. Shawar, K. R. Folger, and C. K. Stover.** 1999. Characterization of a *Pseudomonas aeruginosa* efflux pump contributing to aminoglycoside impermeability. Antimicrob. Agents Chemother. **43:**2975–2983.
43. **Yoneda, K., H. Chikumi, T. Murata, N. Gotoh, H. Yamamoto, H. Fujiwara, T. Nishino, and E. Shimizu.** 2005. Measurement of *Pseudomonas aeruginosa* multidrug efflux pumps by quantitative real-time polymerase chain reaction. FEMS Microbiol. Lett. **243:**125–131.
44. **Ziha-Zarifi, I., C. Llanes, T. Köhler, J.-C. Pechère, and P. Plésiat.** 1999. In vivo emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. Antimicrob. Agents Chemother. **43:**287–291.